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Review Article

The interplay between hepatocyte nuclear factor 4α (HNF4α) and cholesterol sulfotransferase (SULT2B1b) in hepatic energy homeostasis☆

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ABSTRACT

The nuclear receptor hepatocyte nuclear factor 4alpha (HNF4α) plays a critical role in the regulation of metabolic homeostasis, including glucose homeostasis. Sulfotransferases (SULTs) catalyze the transfer of a sulfate group from 3-phosphoadenosine 5-phosphosulfate (PAPS) to an acceptor molecule. Sulfonation plays an essential role in regulating the chemical and functional homeostasis of endogenous and exogenous molecules. Among SULTs, the cholesterol sulfotransferase 2B1b (SULT2B1b) preferentially catalyzes the sulfoconjugation of cholesterol and oxysterols to form cholesterol sulfate and oxysterol sulfates. Hepatic gluconeogenesis represents a critical component of energy metabolism. Although there have been reviews on the regulation of glucose homeostasis by HNF4α, the interplay between HNF4α and SULT2B1b in hepatic glucose homeostasis remains scattered. In this review, we intend to provide an overview on how HNF4α functionally cross-talks with SULT2B1b to regulate hepatic glucose homeostasis and whether the HNF4α-SULT2B1b axis represents a novel therapeutic target for the management of metabolic liver disease and metabolic syndrome.

1. Introduction of hepatocyte nuclear factor 4alpha (HNF4α)

1.1. HNF4α is a nuclear receptor transcriptional factor

Nuclear receptors are a group of ligand binding transcriptional factors. Through their regulation of the expression of target genes, nuclear receptors can regulate a wide array of physiological processes, including energy metabolism.[1] HNF4α, which is also known as NR2A1 (nuclear receptor subfamily 2, group A, member 1), is a nuclear receptor encoded by the *HNF4A* gene.[2,3] HNF4α binds to deoxyribonucleic acid (DNA) as a homodimer.[1] It has been reported that alternative splicing and/or usage of two promoters (P1 and P2) can result in 9 isoforms of HNF4α, including 6 "adult" isoforms (HNF4α1–HNF4α6) from the P1 promoter, as well as 3 "fetal" isoforms (HNF4α7–HNF4α9) from the P2 promoter. The 6 "adult" HNF4α isoforms become abundant after birth, while the 3 "fetal" HNF4α isoforms are expressed throughout liver development but will disappear postnatally.[4] Interestingly, HNF4α1 inhibits the activation of the P2 promoter, which indicates that the dynamic changes in HNF4α isoform expression may be self-regulated by HNF4α1.[5] The transactivation properties of adult and fetal HNF4α isoforms are also different. HNF4α1 is more efficient to activate genes involved in liver development, whereas HNF4α7 plays a more essential role in activating the transcription of early hepatocyte genes.[6] Targeted deletion of the HNF4α1 isoform in the mouse liver results in liver steatosis and marked down-regulation of constitutive androstane receptor (CAR), a key xenobiotic receptor.

1.2. Post-transcriptional modification of HNF4α

A number of post-transcriptional modifications of HNF4α have been reported. HNF4α can be phosphorylated at serine 142 and 143 by protein kinase A (PKA), although HNF4α also has other phosphorylation sites, such as lysine-23 and Y286 by c-Src, serine 87 by protein kinase C (PKC), serine 167 by p38, and serine 313 by adenosine monophosphate-activated protein kinase (AMPK).[7] The

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phosphorylation of HNF4α at serine 142 and 143 by PKA may play a role in HNF4α induction during fasting in a gene- and cell-context dependent manner.[8]

Acetylation plays an essential role in HNF4α mediated gluconeogenesis. 3′, 5′-cyclic adenosine monophosphate (cAMP) response element (CRE)-binding protein (CREB)-binding protein (CBP) possesses an intrinsic acetyltransferase activity capable of acetylating HNF4α at lysine residues within the nuclear localization sequence. CBP-mediated acetylation is crucial for the proper nuclear retention of HNF4α, which is otherwise transported out to the cytoplasm via the export factor chromosome maintenance factor 1 (CRM1) pathway. Acetylation also increases the DNA binding activity of HNF4α.[9] During fasting, pancreas will release glucagon to help keep blood glucose level in the normal range. Glucagon will bind to the glucagon receptor on the cell surface of hepatocytes and subsequently triggers the conformational change of G protein, which results in the dissociation of α-subunit from the G protein complex. Free α-subunits subsequently bind to adenylate cyclase, catalyzing the conversion of adenosine triphosphate (ATP) into cAMP. Two cAMP molecules bind to each regulatory subunit of protein kinase (PKA), releasing its catalytic subunit, which translocates into the nucleus and phosphorylates the CREB at the Ser133 residue. The phosphorylated CREB recruits CBP to the peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α) promoter and regulates its expression. PGC-1α will then coactivate HNF4α and forkhead box protein O1 (FOXO1), and therefore control the transcription of the rate-limiting gluconeogenic enzymes, such as phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase). Deacetylase also plays a role in the homeostasis of acetylation of HNF4α. For example, sirtuin 1 (SIRT1) is a member of a class of oxidized nicotinamide adenine dinucleotide (NAD^+)-dependent deacetylases that adenosine diphosphate (ADP)-ribosylates acetyl lysine protein substrates to remove acetate. SIRT1 can deacetylate HNF4α and inhibit its binding to the promoter of the *PEPCK* gene.[10]

1.3. Role of HNF4α in glucose and lipid metabolism

The role of HNF4α in hepatic glucose and lipid metabolism has been well studied and reported. The HNF4α/PGC-1α pathway plays a crucial role in the transcriptional regulation of hepatic gluconeogenic enzymes such as PEPCK and G6Pase, genes that are activated at fasting and suppressed in a fed state.[11,12] HNF4α and its coactivator, PGC-1α, have been shown to activate the expression of PEPCK and G6Pase by binding to HNF4α-binding cis-elements in their gene promoters.[11,12] The role of HNF4α in hepatic gluconeogenesis has been confirmed by analysis of liver-specific *Hnf4α* knockout mice.[13] HNF4α has also been reported as a target to inhibit gluconeogenesis. For instance, sterol regulatory element-binding protein 1c (SREBP1c) functions as a negative regulator of gluconeogenesis by interacting with HNF4α and interfering with PGC-1 recruitment to suppress hepatic gluconeogenic genes.[14] In addition to positively regulating hepatic gluconeogenesis, HNF4α also plays an essential role in glucose stimulated insulin secretion by pancreatic β cells. Research data has shown that insulin secretion and the intracellular Ca^{2+} response to glucose or tolbutamide were decreased in isolated mutant HNF4α islets. In addition, the responsiveness of the K_{ATP} channel current density to high glucose was decreased in *Hnf4α* knockout mice.[15]

HNF4α is known to be indispensable for maintaining normal triglyceride and cholesterol homeostasis. Loss of hepatic HNF4α results in severe lipid disorder as a result of dysregulation of multiple genes involved in hepatic cholesterol and triglycerides metabolism. Deficiency of HNF4α causes hypotriglyceridemia and the development of fatty liver via reducing very low-density lipoprotein (VLDL) secretion. Moreover, loss of HNF4α results in hypocholesterolemia through several molecular consequences inducing reduction of *de novo* cholesterol biosynthesis, VLDL secretion and high-density lipoprotein (HDL) biosynthesis. Loss of hepatic HNF4α results in low blood triglyceride and cholesterol levels, fatty liver and hepatomegaly. These changes are associated with reduced lipogenesis, *de novo* cholesterol synthesis and VLDL secretion. The messenger ribonucleic acid (mRNA) levels of fatty acid synthase (FAS), diacylglycerol acetyltransferase 1 (DGAT1) and DGAT2 were reduced in *Hnf4α* deficient mice, although the levels of SREBP1c, acetyl-CoA carboxylase (ACC) and steroyl CoA desaturase 1 (SCD1) were unaffected. At the same time, over-expression of HNF4α in the liver moderately lowers plasma cholesterol levels but has no effect on plasma triglyceride levels. Indeed, over-expression of HNF4α had no significant effect on SREBP-2, 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR), SREBP1c, or FAS, suggesting that augmentation of hepatic HNF4α activity may not affect lipogenesis.[16] Studies in human subjects and mice showed that hepatic HNF4α expression is markedly reduced in non-alcoholic steatohepatitis (NASH) patients, diabetic mice, and high-fat diet (HFD)-fed mice as a result of increased micro-RNA-34a expression.[17] Furthermore, HNF4α could directly bind and regulate the expression of hepatic carboxyl esterase 2 (CES2),[18,19] a newly identified triglyceride hydrolase that regulates hepatic lipolysis, endoplasmic reticulum (ER) stress, and lipogenesis. The HNF4α/CES2 pathway is essential in regulating hepatic lipid metabolisms and plays an important role in hepatic lipid disorders like NASH and non-alcoholic fatty liver disease (NAFLD).[19]

2. Introduction of cholesterol sulfotransferase 2B1b (SULT2B1b)

2.1. Phase II sulfotransferases (SULTs)

There are two groups of enzymes that catalyze the biotransformation of endobiotics and xenobiotics including drugs, which are phases I and II drug metabolizing enzymes. Phase II enzymes also play an important role in the biotransformation of endogenous compounds (endobiotics) and xenobiotics to more easily excretable forms as well as in the metabolic inactivation of pharmacologically active substances.[20] The biotransformation of phase II enzymes is mainly to perform conjugating reactions, including glucuronidation, sulfation, methylation, acetylation, glutathione and amino acid conjugation. In general, the resultant conjugates are more hydrophilic than the parent compounds. Phase II drug metabolizing enzymes are mostly transferases, which include: uridine diphosphate (UDP)-glucuronosyltransferases (UGTs), SULTs, *N*-acetyltransferases (NATs), glutathione *S*-transferases (GSTs) and methyltransferases, such as thiopurine *S*-methyl transferase (TPMT) and catechol *O*-methyl transferase (COMT).[20–22]

Among phase II enzymes, a supergene family of enzymes called SULTs can catalyze the reaction of adding a sulfonate moiety to compounds, often leading to increased water solubility and decreased biological activity of the target compounds. There are 44 cytosolic SULTs that have been identified, including 5 SULT families sharing less than 40% sequence homology with each other.[23] Of these 5 SULT families, the SULT1 and SULT2 families represent the largest and most widely studied families. The SULT2 family enzymes are primarily sulfonate neutral steroids (SULT2A) and sterols (SULT2B). Generally, all members of the SULT2 family display overlapping substrate specificities toward an array of hydroxysteroids and related compounds.[24]

2.2. SULT2B1b

The mouse SULT2B1 hydroxysteroid sulfotransferase has two isoforms, SULT2B1b and the SULT2B1a. These two mouse isoforms are derived from the same gene as a result of an alternative exon 1 and differential splicing. Thus, the two isoforms differ only at their amino termini.[25] SULT2B1b, which is also known as cholesterol sulfotransferase, is quantitatively the predominant hydroxysteroid sulfotransferase expressed in the human skin. The human fetal brain appears to only express the SULT2B1a isoform, which is consistent with the evidence that the brain and spinal cord in mice almost exclusively express SULT2B1a.[23] SULT2B1b is expressed in multiple tissues, including the liver. Although the mouse liver does not have a high basal expression of SULT2B1b, the hepatic expression of SULT2B1b is highly inducible, such as in response to 1, 4-bis [2-(3, 5-dichloropyridyloxy)] benzene (TCPOBOP), an agonist for CAR.[26] The human SULT2B1b is highly conserved with the mouse SULT2B1b.[27] The human SULT2B1 also has two isoforms, SULT2B1a and SULT2B1b, which are encoded by a single gene as a result of alternative transcription initiation and alternative splicing. SULT2B1b catalyzes the sulfonation of 3β-hydroxysteroid hormones and cholesterol, whereas SULT2B1a preferentially catalyzes pregnenolone sulfonation.[28]

2.3. Substrates of SULT2B1b

The mouse SULT2B1a shows a distinct preference for pregnenolone as its substrate, whereas cholesterol is only minimally sulfonated by SULT2B1a. In contrast, the mouse SULT2B1b primarily sulfonates cholesterol (Fig. 1), whereas pregnenolone is less avidly metabolized by SULT2B1b.[25] The cholesterol sulfation is a reversible reaction, and the desulfation of cholesterol sulfate is catalyzed by the steroid sulfatase (STS) (Fig. 1). In addition to catalyzing the sulfoconjugation of cholesterol, SULT2B1b can also sulfate specific species of oxysterols, a class of cholesterol derivatives that exhibit broad biological effects ranging from cytotoxicity to the activation of nuclear receptors. SULT2B1b sulfates cholesterol and oxysterols on 3β-hydroxyl group.[29] The human SULT2B1b also prefers cholesterol as its substrate.[27]

2.4. Cholesterol sulfate and its biological functions

Cholesterol sulfate is the primary enzymatic product of SULT2B1b and one of the most predominant steroid sulfates in the circulation. The concentration of cholesterol sulfate in human plasma ranges from 134 to 254 μg/mL.[30,31] Cholesterol sulfate is also present in various body fluids and tissues, including urine, bile, seminal plasma, skin, adrenal, kidney and liver.[30,31] Despite its prevalence and abundance, the physiological role of cholesterol sulfate remains to be defined. Cholesterol sulfate has been recognized to be essential in skin development as a regulatory molecule in the human keratinocyte differentiation and the creation of the barrier.[32] *In vitro* studies showed that cholesterol sulfate is a nature agonist for the retinoic acid-related orphan receptor (ROR).[33] It was suggested that cholesterol sulfate may play a role in immune response, and a shortage of cholesterol sulfate in fetus may contribute to the development of autism.[34] Cholesterol sulfate is also a major cell surface substance that is essential for the cell membrane function.[35]

3. Interplay between HNF4α and SULT2B1b in glucose homeostasis

The liver is an essential organ to maintain metabolic homeostasis. The hepatic glucose metabolism plays an essential role in maintaining energy homeostasis, disruption of which might trigger various metabolic diseases, such as type 2 diabetes, obesity and insulin resistance. HNF4α is highly expressed in the liver. Although the basal expression of SULT2B1b in the liver is not high, it is inducible in mouse models of obesity and type 2 diabetes,[36] suggesting that the expression and/or regulation of SULT2B1b has a role in energy homeostasis.

During prolonged fasting, hepatic gluconeogenesis is the primary source of endogenous glucose production. The whole-body glucose homeostasis is achieved through an intricate balance between glucose production, mostly by the liver, and glucose uptake by the peripheral tissues.[37] Hepatic gluconeogenesis is a major component of hepatic glucose production, the rate of which is controlled by the rate-limiting gluconeogenic enzymes PEPCK and G6Pase.[38] The *PEPCK* and *G6Pase* genes are positively regulated by cAMP, glucocorticoids and glucagon, whereas the same genes are negatively regulated by insulin.[39,40] HNF4α is a liver-enriched orphan nuclear receptor that plays a pivotal role in energy homeostasis by regulating the metabolism of glucose and lipids. HNF4α promotes gluconeogenesis through its positive regulation of *PEPCK* and *G6Pase* genes.[41,42] Although the HNF4α-promoted gluconeogenesis is physiologically essential, uncontrolled gluconeogenesis is harmful and represents a major pathogenic event in the development of type 2 diabetes.[43,44]

3.1. SULT2B1b and cholesterol sulfate inhibit gluconeogenesis by targeting HNF4α

We recently reported that the expression of SULT2B1b in the liver was induced in obese mice and during the transition from the fasting to the fed state, suggesting that the regulation of SULT2B1b is physiologically relevant.[36] *In vitro*, overexpression of SULT2B1b in both human and mouse hepatocytes decreases glucose production and gluconeogenic gene expression, which is explained by the enzymatic activity of SULT2B1b, because overexpression of an enzymatic dead mutant SULT2B1b failed to inhibit glucose production. Moreover, treatment of hepatocytes with cholesterol sulfate, the enzymatic byproduct of SULT2B1b, also suppressed the glucose production and expression of gluconeogenic enzymes.[36]

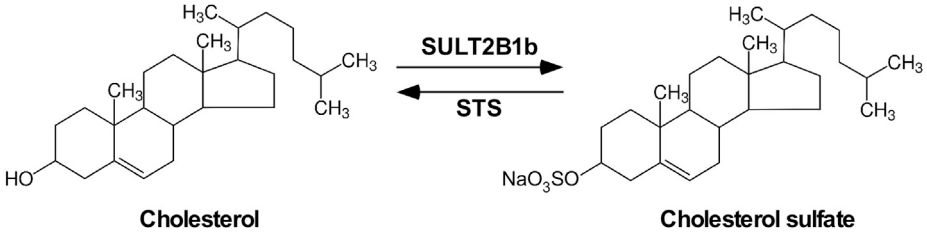

Fig. 1. Schematic representation of cholesterol sulfation and desulfation of cholesterol sulfate. The SULT2B1b catalyzes the sulfation of cholesterol to form cholesterol sulfate, whereas the STS catalyzes the desulfation of cholesterol sulfate. Abbreviations: SULT2B1b, cholesterol sulfotransferase 2B1b; STS, steroid sulfatase.

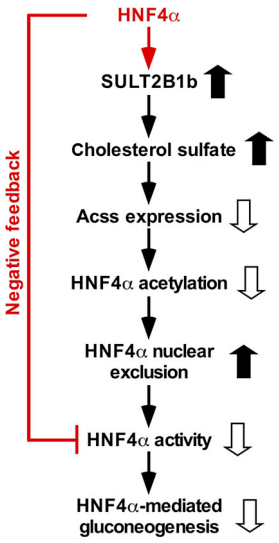

Fig. 2. The interplay between HNF4α and SULT2B1b in glucose homeostasis. The key components of this regulatory system include: (i) SULT2B1b and cholesterol sulfate inhibit gluconeogenesis by targeting nuclear receptor HNF4α; and (ii) regulation of SULT2B1b by HNF4α constitutes a negative feedback control of hepatic gluconeogenesis. Abbreviations: HNF4α, hepatocyte nuclear factor 4alpha; SULT2B1b, cholesterol sulfotransferase 2B1b; Acss, acetyl-CoA synthetase.

Further analysis showed that cholesterol sulfate and SULT2B1b inhibited gluconeogenesis by targeting HNF4α in hepatocytes treated with cholesterol sulfate or overexpressing SULT2B1b. The inhibitory effects of cholesterol sulfate and SULT2B1b on HNF4α were also shown in SULT2B1b transgenic mice that overexpress SULT2B1b in the liver. Cholesterol sulfate and SULT2B1b specifically inhibit HNF4α-mediated gluconeogenesis but have little effect on the gluconeogenic activity of PGC-1α and FOXO1. Treatment of mice with cholesterol sulfate or transgenic overexpression of SULT2B1b in the liver inhibits hepatic gluconeogenesis and alleviates metabolic abnormalities in both the HFD-induced obesity and *ob/ob* mice. Mechanistically, cholesterol sulfate and SULT2B1b inhibit gluconeogenesis by suppressing the expression of acetyl-CoA synthetase (Acss), leading to decreased acetylation and nuclear exclusion of HNF4α. As summarized in Fig. 2, it was concluded that SULT2B1b and its enzymatic byproduct cholesterol sulfate are important metabolic regulators that control glucose metabolism, suggesting cholesterol sulfate as a potential therapeutic agent and SULT2B1b as a potential therapeutic target for metabolic disorders.

Although cholesterol sulfate was shown to be effective to inhibit gluconeogenesis *in vitro* and *in vivo*, cholesterol sulfate is readily hydrolyzed by the STS (Fig. 1), limiting its utility as a therapeutic agent. In an effort to improve the chemical stability of cholesterol sulfate, we have recently reported the development of thiocholesterol, a structural analog of cholesterol sulfate generated by replacing the sulfate with a thiol group (Fig. 3), which is predicted to be hydrolysis resistant.[45] Thiocholesterol exhibited an improved intracellular stability and better efficacy in inhibiting gluconeogenesis in primary mouse and human hepatocytes. Moreover, thiocholesterol showed a more superior activity in reducing fasting blood glucose levels and improving overall glucose homeostasis in HFD-fed mice, which was attributed to the increased stability and bioavailability of thiocholesterol.[45]

3.2. Regulation of SULT2B1b by HNF4α constitutes a negative feedback control of hepatic gluconeogenesis

Having shown that SULT2B1b inhibits hepatic gluconeogenesis by antagonizing the gluconeogenic activity of HNF4α, it was rather a surprise for us to find that SULT2B1b itself is a transcriptional target of HNF4α.[45] Transfection of HNF4α induces the expression of SULT2B1b in both mouse and human hepatocytes, as well as in the mouse liver. HNF4α regulates *SULT2B1b* gene expression through its binding to the promoter regions of both the human and mouse *SULT2B1b* genes. The establishment of *SULT2B1b* as an HNF4α target gene led to our hypothesis that the induction of SULT2B1b by HNF4α represents a negative feedback to limit the gluconeogenic activity of HNF4α.[45] In this model, on the one hand, the gluconeogenic activity of HNF4α is inhibited by SULT2B1b. On the other hand, HNF4α upregulates the expression of SULT2B1b, limiting its own gluconeogenic activity. Consistent with this hypothesis, small interfering RNA (siRNA) down-regulation or knockout of *Sult2B1b* enhanced the gluconeogenic activity of HNF4α *in vitro* and *in vivo* as a result of loss of the negative feedback (Fig. 2).

Mechanistically and consistent with the observations made in SULT2B1b gain of function models, the enhanced gluconeogenic activity of HNF4α in *SULT2B1b* knockdown or knockout hepatocytes or livers was associated with the increased acetylation and nuclear enrichment of HNF4α.[45] The increased acetylation of HNF4α in *SULT2B1b*$^{-/-}$ hepatocytes may be explained by the combined effects of a decreased expression of the HNF4α deacetylase Sirt1 and an increased expression of the acetyl-CoA-generating enzyme Acss. The mechanisms by which Sirt 1 and Acss are regulated by Sult2B1b remain to be understood. We conclude that the HNF4α-SULT2B1b-cholesterol sulfate axis represents a key endogenous mechanism to prevent uncontrolled gluconeogenesis (Fig. 2).

3.3. The role of the interplay between HNF4α and SULT2B1b in fasting response

Fasting is known to induce the expression and activity of HNF4α and increase gluconeogenesis as a physiological response. As an endogenous regulatory mechanism to control the hepatic

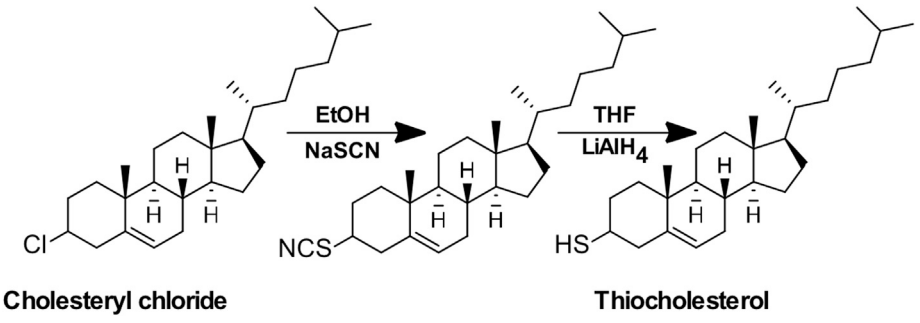

Fig. 3. Schematic representation of the chemical reactions to synthesize thiocholesterol from cholesteryl chloride. Abbreviation: EtOH, ethanol.

gluconeogenesis, the interplay between HNF4α and SULT2B1b also plays a role in the control of fasting blood glucose levels. The fasting-responsive expression of G6Pase and PEPCK is elevated in *Sult2B1b*$^{-/-}$ mice compared to their wild type counterparts. Moreover, the *Sult2B1b*$^{-/-}$ mice exhibited a higher level of fasting glucose, which may contributed to the increased gluconeogenesis.[45]

4. SULT2B1b in lipid metabolism

Hepatic lipid metabolism involves the synthesis of lipids (lipogenesis), β-oxidation and lipid secretion. Lipogenesis is the *de novo* fat synthesis, including fatty acid synthesis and subsequent conversion of fatty acids to triglycerides in the liver and adipose tissue. Uncontrolled hepatic lipogenesis will lead to steatosis (fatty liver) caused by excessive triglyceride accumulation. There are several enzymes directly involved in lipogenesis, including FAS, ACC1 and SCD1, all of which are under the transcriptional control of SREBP1c.[46]

4.1. The cross-talk between the regulation of HNF4α and SULT2B1b with liver X receptor (LXR) in hepatic lipogenesis

In addition to the inhibition of gluconeogenesis, SULT2B1b also has an anti-lipogenic activity.[47] Mice overexpressing SULT2B1b exhibit better metabolic functions, which is in part contributed by the anti-lipogenic activity of SULT2B1b.[36] Both SULT2B1b overexpression and treatment with cholesterol sulfate or its chemical derivative thiocholesterol resulted in the improvement on hepatic steatosis and a decrease in the expression of SREBP1c in mice.[45] The downregulation of SREBP1c may have accounted for the suppression of the *Acss* gene, an SREBP1c target gene.[55] Moreover, adenoviral overexpression of *SULT2B1b* suppressed the LXR- and SREBP1c-mediated lipogenic pathways,[48] which might be due to the sulfation and deactivation of oxysterols, the endogenous LXR agonists.[48] Adenoviral overexpression of *Sult2B1b* in the mouse liver also decreased the SREBP1c protein level.[48] On the other hand, SREBP1c competitively inhibited PGC-1α recruitment, a requirement for HNF4α activation. Consistent with these results, hepatic *Pepck* and *G6pase* mRNA levels were suppressed by overexpression of *SREBP1a* and *SREBP1c* in the transgenic mice. This result indicates that SREBP1 may act as a negative regulator of gluconeogenic genes through a cross-talk with HNF4α interference with PGC-1α recruitment. Since the expression of *SULT2B1b* is positively regulated by HNF4α, the cross-talk between HNF4α, SULT2B1b and SREBP1c may constitute a novel mechanism to help maintain the hepatic lipogenic level.

4.2. The cross-talk between the regulation of HNF4α and SULT2B1b with CAR in hepatic lipogenesis

CAR is one of the key xenobiotic receptors that regulates hepatic expression of a large number of drug-processing genes (DPGs).[49] As a xenobiotic receptor, CAR inhibits hepatic lipogenesis through downregulating the expression of lipogenic LXR target genes, partially by upregulating the expression of its downstream target gene *SULT2B1b*. HNF4α transactivates the hepatic expression of *CAR*,[50] and HNF4α synergizes with *CAR* to induce CAR-target DPGs.[51,52] *SULT2B1b* is reported to be the downstream target gene of CAR, which indicates that in addition to direct binding to the promoter of the *SULT2B1b* gene, HNF4α positively regulating the expression of *SULT2B1b* may be partially through its coactivation with CAR. Interestingly, there is another functional inhibitory cross-talk between CAR and HNF4α in hepatic lipid/glucose metabolism, that CAR down-regulates HNF4α-target genes through competing for common coactivators and/or competing with HNF4α for binding to the DR1 motif in the promoter of cytochrome P450 (CYP) 7A1, the rate-limiting enzyme in bile acid biosynthesis.[53] Accordingly, TCPOBOP, the CAR activator, decreased the hepatic expression of CYP7A1 and CYP8B1, and increased the hepatic expression of SULT2B1b. This may be caused by the inhibitory effects of SULT2B1b on HNF4α.

4.3. SULT2B1b and ROR in hepatic lipogenesis

The anti-lipogenic activity of SULT2B1b is reported to be associated with an increased level of circulating cholesterol sulfate. Cholesterol sulfate has been reported as a natural agonist of ROR.[33] Mice overexpressing SULT2B1b displayed an increase in hepatic expression of ROR target genes, such as the *Cyp7b1*, aryl hydrocarbon receptor nuclear translocator-like protein-1 *(Bmal1)*, and IκB kinase (*Ikk*) genes.[36] ROR is reported to trans-suppress the lipogenic activity of LXR.[53] This indicates that the increased production of cholesterol sulfate and activation of ROR may represent a novel LXR-mediated mechanism by which SULT2B1b inhibits lipogenesis.

5. Summaries and perspectives

SULT2B1b is a cholesterol sulfotransferase, but its physiological and pathophysiological role in energy homeostasis, especially in gluconeogenesis, is previously under-investigated. Our recent work showed that SULT2B1b and its enzymatic product cholesterol sulfate inhibited gluconeogenesis by targeting the gluconeogenic transcriptional factor HNF4α. Cholesterol sulfate and SULT2B1b inhibit gluconeogenesis by suppressing the acetylation of HNF4α and sequestration of the nuclear localization of HNF4α. Our studies have established cholesterol sulfate as an important metabolic regulator in controlling glucose metabolism and energy homeostasis, pointing to cholesterol sulfate as a potential therapeutic agent and SULT2B1b as a potential therapeutic target for metabolic disorders. This notion was further supported by our development of the hydrolysis-resistant thiocholesterol that shows more superior activity than cholesterol sulfate in inhibiting gluconeogenesis, and improving overall glucose homeostasis in HFD-induced diabetic mice. SULT2B1b was subsequently identified as a novel transcriptional target of HNF4α. We propose that the induction of SULT2B1b by HNF4α constitutes a negative feedback to prevent uncontrolled gluconeogenesis. Ablation of SULT2B1b increases HNF4α-mediated glucose production in hepatocytes and the fasting blood glucose *in vivo*.

In addition to its activities in inhibiting gluconeogenesis and lipogenesis, SULT2B1b has been reported to have many other functions in different cell types and tissues, ranging from promoting liver cell proliferation to affecting prostate and colorectal cancer cells, and inhibiting the T cell receptor signaling.[54–56] It will be interesting to know whether HNF4α, as a positive regulator of SULT2B1b, is also involved in these additional functions of SULT2B1b. In the meantime, HNF4α has many functions besides gluconeogenesis and lipogenesis.[57] It remains to be determined whether and how *SULT2B1b*, as an HNF4α target gene, may have contributed to other cellular functions of HNF4α.

Last but not least, SULT-mediated sulfonation is a reversible reaction. The desulfation of cholesterol sulfate is catalyzed by STS, a desulfation enzyme that converts hormonally steroid sulfates, such as cholesterol sulfate, androgen sulfates and estrogen sulfates, to hormonally active steroids. STS has also been reported to play a tissue and sex specific role in controlling energy homeostasis. These include: (i) our recent report showing that hepatic overexpression of STS ameliorates mouse models of obesity and type 2 diabetes

through sex-specific mechanisms.[58] Results from this study suggested that STS mediated estrogen re-activation in the liver is beneficial in energy and glucose metabolism, and liver-specific activation of estrogen signaling may represent a novel approach to manage metabolic syndrome; and (ii) the sex-dimorphic and sex hormone-dependent role of STS in adipose inflammation and energy homeostasis.[59] Transgenic overexpression of STS in the adipose tissue of male mice exacerbated the HFD-induced metabolic phenotypes, including increased adipose inflammation, which may have been accounted for by increased androgen activity in the adipose tissue, and castration abolished most of the phenotypes. Interestingly, the transgenic effects were sex-specific, because the HFD-fed female STS transgenic mice exhibited improved metabolic functions, which were associated with increased estrogenic activity in the adipose tissue and attenuated adipose inflammation. These results suggested that the adipose STS may represent a novel therapeutic target for the management of obesity and type 2 diabetes. It is conceivable that the balance between SULT2B1b-mediated cholesterol sulfation and STS-mediated desulfation of cholesterol sulfate is important to fine tune the energy homeostasis.

Authors' contributions

Y. Bi: study concept and design; drafting of the manuscript. Y. Wang: critical revision of the manuscript. W. Xie: study concept and design; critical revision of the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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